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BOTANICAL GAZETTE

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CHEMICAL STIMULATION AND THE EVOLUTION OF CARBON DIOXID.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XLIV.

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(WITH TWO FIGURES)

SEVERAL years ago, in a study by Kahlenberg and myself¹ of the effect of placing small pieces of various chemical elements in water where seedlings were growing, it was found that in a considerable number of instances the roots of the seedlings exhibited an acceleration of growth, which we construed as an effect of the chemical employed. In individual cases we found a rapid elongation of the primary root, probably resulting from the action of Bi, Cr, In, Se, Te, Hg, Bo, Pb, W, Co, and Cu. This abnormally rapid growth was frequently followed promptly by death. As the compounds of these elements began to enter into solution, their first action on the plant was that of stimulants; when these same compounds became more concentrated, they became distinctly, often fatally, toxic.

The stimulating action of minute quantities of various poisons has long been known in the case of a few fungi. Raulin² found that salts of Zn, Fe, and Mn, as well as silicates, caused an unusually luxuriant growth of *Aspergillus*. Richards³ extended

¹ COPELAND, E. B., and KAHLENBERG, LOUIS, The influence of the presence of pure metals upon plants. Trans. Wisc. Academy 12:454-474. 1899.

² RAULIN, J., Études chimiques sur la végétation. Ann. Sc. Nat. Bot. V. 11:93-299. 1869.

³ RICHARDS, H. M., Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. 30:665-688. 1897.

Raulin's observations, using *Aspergillus*, *Penicillium*, and *Botrytis* as subjects, and Zn-, Fe-, Co-, and Ni-sulfates, NaFl, LiCl, Na₂SiO₃, cocain, morphin, and amygdalin as stimulants. He also recognized the phenomenon in its true light, as one of stimulation. Ono⁴ used almost exclusively the same salts and HgCl₂ in addition, with the same results as Richards. Richter,⁵ from work with a wide range of concentrations, regards the Zn ion as stimulating the growth of *Aspergillus*, but the ZnSO molecule as depressing it, and recognizes no acceleration under the influence of Cu. Pulst⁶ added *Mucor* to the list of subjects, and Cd and Tl to the list of poisons which stimulate growth if sufficiently dilute. Hattori⁷ has also found abnormally active growth of fungi in CuSO₄ solutions, De Seynes⁸ describes a strain of *Penicillium* thriving in it, and Gosio⁹ reports *Mucor*, *Aspergillus*, and another strain of *Penicillium* thriving in the presence of arsenic. Similar observations to Gosio's were made long ago by Preuss¹⁰ and Bouchardat.¹¹ Yasuda¹² finds that the addition of suitable amounts of alkaloids to the nutrient solutions favors the growth of molds, the mode of action, whether as food or stimulant, not being determined.

Turning to plants other than the fungi, a similar stimulating

⁴ONO, N., Notes on the stimulating effect of certain substances upon the growth of algae and fungi. Bot. Mag. Tōkiō 14: 75-78. 1900. Also, Ueber die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sc. Imp. Univ. Tōkiō 13: 141-186. 1900.

⁵RICHTER, A., Zur Frage der chemischen Reizmittel. Centralbl. Bakteriöl. 7: 417-429. 1901.

⁶PULST, C., Die Widerstandsfähigkeit einiger Schimmelpilze gegen Metallgifte. Jahrb. Wiss. Bot. 37: 205-263. 1902.

⁷HATTORI, Studien über die Einwirkung des Kupfersulfats auf einige Pflanzen. Jour. Coll. Sc. Imp. Univ. Tōkiō 15: 371-394. 1901.

⁸DE SEYNES, J., Resultats de la culture du *Penicillium cupricum* Trabut. Bull. Soc. Bot. France 42: 451-455, 482-485. 1895. [Bot. Centralbl. 66: 157.]

⁹GOSIO, B., Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bedingt wird. Ber. Deutsch. Chem. Ges. 30: 1024-1026. 1897.

¹⁰PREUSS, Wirkung des Arsens auf Vegetabilien. Bot. Zeit. 6: 409-412. 1848.

¹¹BOUCHARDAT, Recherches sur la végétation appliquées à l'agriculture. Paris, 1846. Rev. in Bot. Zeit. 5: 85, 102. 1847.

¹²YASUDA, A., On the effect of alkaloids upon some moulds. Bot. Mag. Tōkiō 15: 79-83. 1901.

action of dilute poisons, well established in those organisms physiologically nearest them, the bacteria, led Hueppe¹³ to the formulation of this "biologischen Grundgesetz:"

Jeder Körper, der in bestimmter Concentration Protoplasma tödtet und vernichtet, in geringeren Mengen die Entwicklungsfähigkeit aufhebt, aber in noch geringeren Mengen, jenseits eines Indifferenzpunktes, umgekehrt als Reiz wirkt und die Lebenseigenschaften erhöht.

Ono (*l. c.*) detected an acceleration of the growth of algae by all the same substances to which his fungi responded, except Hg; but found the optimum concentration much lower, corresponding to the greater susceptibility of the alga to injury by these poisons.

Our information on the chemical stimulation of the growth of phanerogams is altogether fragmentary. The most familiar example of this is the exceptionally luxuriant growth of violets and some other plants in the neighborhood of zinc works. Rumm¹⁴ finds that in grape vines sprayed with copper various leaf tissues become better developed, and the fruit ripens earlier. Berlese and Sostegni¹⁵ substantiate this, but seem to believe that the most important immediate effect of the copper is the increase of the cyanophyll, other effects being results of this. Frank and Krüger¹⁶ show that potatoes sprinkled with copper solutions excel in leaf area, length of life, and amount of crop. Townsend finds that the growth of *Avena* seedlings is accelerated at least temporarily by not too much ether in the atmosphere;¹⁷ that the germination of various seeds and spores may be hastened

¹³ HUEPPE, F., *Naturwissenschaftliche Einführung in die Bakteriologie*. Wiesbaden. 1896. Hueppe gives Arndt and Schultz joint credit with himself for this generalization.

¹⁴ RUMM, C., *Ueber die Wirkung der Kupferpräparate bei Bekämpfung der sogenannten Blattkrankheit der Weinrebe*. *Ber. Deutsch. Bot. Gesells.* 11:79-93. 1893.

¹⁵ BERLESE et SOSTEGNI, *Recherches sur l'action des sels de cuivre sur la végétation de la vigne et sur le sol*. *Rev. internat. de Vitic. et Oenologie*. 1895. [*Bot. Jahresber.* 23¹:292-294. 1895.]

¹⁶ FRANK, B., und KRÜGER, F., *Ueber den Reiz welchen die Behandlung mit Kupfer auf die Kartoffel hervorruft*. *Ber. Deutsch. Bot. Gesells.* 12:8. 1894.

¹⁷ TOWNSEND, C. O., *The correlation of growth under the influence of injuries*. *Ann. Botany* 11: 509-532. 1897.

by weak enough ether,¹⁸ and that germination and subsequent growth can be accelerated by a short exposure to HCN gas.¹⁹ Lövinson²⁰ discovered that germination was hastened by CS₂. De Toni and Mach²¹ are said to get the same result by treating tobacco seeds with alkaloids; Windisch²² finds that Ca(OH)₂, though damaging to some kinds of seeds, improves the germination of oats; and Böttger²³ has hastened the germination of coffee with dilute KOH. Kahlenberg and True²⁴ found that in acid solutions their seedlings had a tendency toward abnormally rapid growth, followed by death; but experiments on this point with HCl yielded only negative results. An unsigned note²⁵ in an old Flora says:

Bekanntlich können Samen, welche schwer keimen, oder die Fähigkeit dazu bereits verloren haben, dadurch zum Keimen gebracht werden, dass man dieselben längere Zeit in mit Salzsäure schwach angesäuertes Wasser legt.

This is followed by a review of work, the original publication of which in "Silliman's Journal" I have not located, on seeds treated with very dilute HCl. *Lactuca sativa* in these experiments was two and one-half inches high in 48 hours, and in eight days had reached the state of development normally requiring five or six weeks; and young pines and firs grew so, that, at the end of three months, they were taken for two years old.

¹⁸ TOWNSEND, C. O., The effect of ether upon the germination of seeds and spores. BOT. GAZ. 27:458-466. 1899.

¹⁹ TOWNSEND, C. O., The effect of hydrocyanic acid gas upon grains and other seeds. BOT. GAZ. 31: 241-264. 1901.

²⁰ LÖVINSON, O., Ueber Keimungs- und Wachstumsversuche an Erbsen in Lösungen von fettsauren Salzen unter Ausschluss von Mineralsäuren. Bot. Centralbl. 83:1, 33, 65, 97, 129, 185, 210. 1900.

²¹ DETONI, B. G., e MACH, P., Sopra l'influenza esercitata della nicotina, etc. Boll. R. Ist. Bot. Univ. Parmense. 1893. Not seen.

²² WINDISCH, R., Ueber die Einwirkung des Kalkhydrates auf die Keimung. Landw. Versuchsst. 54:283-309. [Bot. Centralbl. 87:217.]

²³ BÖTTGER, R., Beförderung des Keimprocesses. Jahresb. phys. Ver. Frankfurt. 1872-3. [Bot. Jahresber. 1:833. 1874.]

²⁴ KAHLENBERG, L., and TRUE, R. H., On the toxic action of dissolved salts and their electrolytic dissociation. BOT. GAZ. 22:81-124. 1896.

²⁵ Beförderung des Wachstums der Pflanzen durch Salzsäure. Flora 28:464. 1845.

Immediately following the before-mentioned work by Kahlenberg and myself in the spring of 1899, I started a series of water cultures in which Zn and Cu were present. The results were such as to encourage a continuance of the work, and showed that the acceleration of growth is most decided by solutions not a great deal more dilute than those that are distinctly toxic. In fact, the concentrations producing the two effects differed by less than the individual variations of the seedlings used, so that, as in our earlier work when the metals themselves were placed in the water, it sometimes happened that in the same culture (vessel) some plants were evidently injured, while others grew exceptionally well. It is evidently reasonable, even if out of accord with our usual way of looking at it, to regard the acceleration of growth as itself an injury to the plant.

In the summer of 1899 appeared Jacobi's²⁶ work on the influence of various substances on the respiration and assimilation of water plants. I was immediately impressed with the superiority of respiration to growth as a true index of the plant's activity. I need not enter into a discussion of the relation of respiration to the plant's multifarious other manifestations of activity. It makes available the energy for most of them; without it all would cease. Growth is dependent on respiration, and under ordinary conditions they vary together; when they do not, it is respiration which reveals the plant's real activity. From the minimum to the optimum temperatures for growth, its curve rises with that of respiration, the more rapid combustion in the plant furnishing more energy and proper material for growth. But the more active the respiration, the less the *relative* amount of plastic material left available for growth. A point must be reached, then, beyond which an increase in respiration can reasonably be regarded as itself the cause of a decrease in growth. This is what happens at temperatures above the optimum for growth. How far this loss of material can go during the most rapid respiration is shown by Kraus's²⁷ classic work, in

²⁶ JACOBI, B., Ueber den Einfluss verschiedener Substanzen auf die Athmung und Assimilation submerser Pflanzen. *Flora* **86**: 289-327. 1899.

²⁷ KRAUS, G., Ueber die Blütenwärme bei *Arum italicum*. *Abhandl. Naturf. Gesells. Halle* **16**:—. 1884. Also, Ueber Blütenwärme bei Cycadeen, Palmen und Araceen. *Ann. Jard. Bot. Buitenzorg* **13**: 217-275. 1896.

which he found as much as three-fourths of the substance of some spadices consumed within a few hours; yet respiration need not use up the combustible material at any rate comparable to this to make constructive growth impossible.

Exactly the same phenomenon should be anticipated when respiration is accelerated by a chemical stimulus as when heat is the agent. The result of moderate stimulation will be a quickening of the growth, but with a more violent stimulation of the respiration, growth will be hindered and ultimately stopped. Wherever my work has thrown any light on this question, it has made this view seem more certainly the correct one. The more concentrated the solutions used, the more is the respiration accelerated, even when the concentration is fatal in twenty-four hours or less. In the isolated case in my experiments, in which with increasing concentration there was an initial decrease in the respiration, the solution was so strong as to plasmolyse the cells violently, and the result was without doubt due to the osmotic (physical) action. In the relatively few instances in which it has been sought, an optimum concentration for vegetative growth has always been determined, and the known antagonistic effect of considerably greater concentrations than those accelerating growth shows that an optimum must exist for each stimulant and plant. An optimum concentration of a chemical stimulant for respiration is not known in a single instance; it does not exist.

The "biologische Grundgesetz" of Huetpe is not fundamental. It applies only to growth, or to growth and other phenomena standing in the same relation with it to respiration. It means merely that when respiration is not already too active, accelerating it will result in a quicker growth. It is indifferent whether the accelerating agent be chemical or thermal. The explanation I have offered of the opposite effects on growth of dilute and more concentrated solutions would do away entirely with Richter's idea that the difference is because ions stimulate, but molecules poison. As already stated, no difference exists except in degree between solutions of different strength in their action on respiration. Richter's explanation is difficult to

harmonize with the details of the degrees of dissociation, since dissociation is very nearly complete in fatal concentrations of several poisons (Kahlenberg and True, 1896). And it fails utterly to explain the rather abrupt change from accelerating to obviously toxic concentrations. This quick change has, from my point of view, a perfect analogue in the plant's thermal relations in the steep fall of the curve of growth above the optimum temperature. Even the ion theory can be overworked.

If we interpret the action of chemical stimuli on growth as secondary to that on respiration, we are already armed with an explanation of their influence on reproduction. Klebs²⁸ has shown that the demands of reproduction upon various conditions are more strict than those of growth. This may be regarded as meaning, in part at least, that the balance of available plastic matter must be relatively great, while the sacrifice of matter for energy must be less than is tolerable for growth. The optimum temperature for reproduction would then be sought below that for growth, and a chemical stimulus accelerating growth might inhibit reproduction. From Klebs²⁹ again, we know that while the optimum for growth of *Vaucheria repens* is 30° C., the maximum for the formation of zoospores (p. 46) and for sexual reproduction (p. 112) is about 26° C. This idea might still be sound, even if it should be shown that the least perceptible quantity of a poison interfered with reproduction. Of course I offer this only as a suggestion, and do not overlook the very likely alternative that acceleration of the growth is the factor which in its turn injures the reproduction. Of course, too, there are outside factors which act on the growth quite independently of the respiration. The blue end of the solar spectrum slackens growth, but has no well authenticated direct effect on respiration. In the case of all chemical stimuli, however, so far as they are known at all, I hold it more than probable that their apparent effect on growth is a function of their more direct influence on respiration.

²⁸ KLEBS, G., Einige Ergebnisse der Fortpflanzungs-Physiologie. Ber. Deutsch. Bot. Gesells. 18:(201)-(215). 1900.

²⁹ KLEBS, G., Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.

I have intercalated this discussion at this point, in advance of the evidence on which my opinions are partly based, in order that the primary position of respiration in the plant's economy, and its eminent fitness as a field of study in chemical irritability might be clearly in mind. The importance of respiration will not be at all impaired if the view well maintained in animal physiology by Loeb and others, which regards the energy relations characteristic of living things as electrical rather than thermal, is found altogether correct.

While there is an extensive literature on the influence on respiration of sugars and other substances which furnish the plant with material to be oxidized, there has been surprisingly little work done with chemicals acting primarily as stimulants. The most considerable collection of such data is that of Jacobi, who tested the effect of KNO_3 , KCl , NaCl , chinin, antipyrin, I , oxalic acid, and CuSO_4 on the respiration of *Elodea* and *Myriophyllum*. Some of these substances were given but a single test, and none of them were tried in any considerable range of concentrations. Previous to Jacobi, Kellner³⁰ had found an acceleration in the respiration of pea seedlings by treating them with KNO_3 , and attributed it to direct oxidation by the O of the saltpeter. Elfving,³¹ Lauren,³² Johannsen,³³ and Morkowine,³⁴ have demonstrated the acceleration of respiration by anesthetics. Morkowine³⁵ has also extended Jacobi's work on the action of alkaloids. Mayer³⁶ reports that HCN reduces the activity of respiration, as

³⁰ KELLNER, O., Ueber einige chemische Vorgänge bei der Keimung von *Pisum sativum*. Landw. Versuchsst. 17: 408. 1874. [Bot. Jahresb. 1: 828. 1874].

³¹ ELFVING, F., Ueber die Einwirkung von Aether und Chloroform auf die Pflanzen. Finska Vetensk. Soc. Förh. 28: 36. 1886. I have not seen this paper.

³² LAUREN, W., Ueber den Einfluss von Aetherdämpfen auf die Athmung von Keimlingen. Helsingfors. Diss. 1891. [Bot. Jahresb. 20¹: 92. 1892.]

³³ JOHANNSEN, W., Aether- und Chloroformnarkose und deren Nachwirkungen. Bot. Centralbl. 68: 337-338. 1896.

³⁴ MORKOWINE, N., Recherches sur l'influence des anesthésiques sur la respiration des plantes. Rev. Gén. Bot. 11: 289-352. 1899.

³⁵ MORKOWINE, N., Recherches sur l'influence des alcaloides sur la respiration des plantes. Rev. Gén. Bot. 13: 109, 177, 212, 265. 1901.

³⁶ MAYER, AD., Ueber den Einfluss der Blausäure auf Pflanzenathmung. Landw. Versuchsst. 23: 335. 1879.

KCN is believed to do in animal physiology. Diakonow³⁷ states that with increasing acidity of the nutrient medium intramolecular respiration of molds decreases, but normal respiration is not sensibly affected. Kosiński³⁸ finds the respiration of *Aspergillus* quickened by Zn, Fe, Mn, cocain, strychnin, and not too concentrated ether; he tested ZnSO_4 as strong as 0.1 per cent. without its depressing the respiration during the first day. He also reports a stimulation by .0005 per cent. ZnSO_4 , but this appearance is quite possibly due to the growth of his material, as appears more probable from his finding the acceleration not very dependent on the concentration of salt applied. Ono (*l. c.* 1900, p. 156) found that the proper concentration of NiSO_4 increased the formation of oxalic acid, but got a similar result with no other poison. The acceleration of fermentation by yeasts, by the presence of traces of poisons, is a very nearly related phenomenon.

In my measurements of respiration I have used a modification of the Pettenkofer method, which has enabled me to work with an assurance of reliable results not possible with apparatus hitherto used. Respiration varies rapidly with changes in temperature, and absolute constancy of temperature is not practicable. The best we have been able to do is to make the variations as slight as possible; and in studying the influence of other factors on respiration, the error from the thermal variations has had to be ignored. Working with water plants this error is augmented by the lesser solubility of CO_2 at higher temperatures. The rate at which air passes through the apparatus has a more than appreciable influence on the results under any conditions; and when working with solutions the solubility of the CO_2 makes glaring the results of small irregularities in the rate of flow. When experiments are lasting longer than a few hours the rate of respiration may change, and the possible error from this source is very imperfectly overcome when, with other plants,

³⁷ DIAKONOW, N. W., Intramolekulare Athmung und Gährthätigkeit der Schimmelpilze. Ber. Deutsch. Bot. Gesells. 4: 2-7. 1886.

³⁸ KOSIŃSKI, I., Die Athmung bei Hungerzuständen und unter Einwirkung von mechanischen und chemischen Reizmitteln bei *Aspergillus niger*. Jahrb. Wiss. Bot. 37: 137-204. 1901.

under conditions which it is hardly possible to make identical, the respiration is found constant.

I had been but a few weeks at this work before irregularities in my results, which I could not prevent, and often could not account for—just such irregularities as cast a suspicion on more than one piece of work on respiration—made it very evident that sound results on respiration are not to be obtained without the same precaution in the use of parallel controls which is regarded as indispensable in other fields of physiology. So far as I know, the only work in which a real control has been used in experiments on respiration is that of Moller,³⁹ whose apparatus is in that respect more worthy of imitation than the widely followed one of Pfeffer and Wilson.⁴⁰

The apparatus which I have used is illustrated by the accompanying diagram (*fig. 1*). The subjects of experiment are submerged in water in a large can. In the early part of the work, I used in place of this can a large stone churn, black inside, with a turned wooden lid soaked in India ink, and plugged the openings around the connections with cotton similarly soaked; but I have since satisfied myself that the light in the interior of the laboratory will not disturb the experiment if no care at all is taken to exclude it from the can. The air comes to both bottles of experiment material after the removal of all CO_2 by passing two towers of solid KOH and a solution of $\text{Ba}(\text{OH})_2$. From each subject of experiment the air passes to a bottle, in whose stopper are four holes, which serves admirably, and without taking more than a minimum of room, as a switch board. When the experiments were carried on at a much higher temperature than that of the room, these bottles contained H_2SO_4 to prevent the dilution of the baryta water by distillation from the experiment bottles. In the diagram the absorption tubes are Winkler coils. I have used them and Pettenkofer tubes about equally, and have no choice, except that with the

³⁹ MÖLLER, H., Ueber Pflanzenathmung. Ber. Deutsch. Bot. Gesells. 2: 306–321. 1884.

⁴⁰ PFEFFER, W., Ueber intramolekulare Athmung. Unters. Bot. Inst. Tübingen 1: 636–685. 1885.

latter it is not easy to keep the glass tube by which the air enters the baryta water free of BaCO_3 . It is not difficult to keep either kind of tube clean if it is always washed, after using, with *dilute* HCl . Using two absorption tubes with each culture, the experiment is continuous, only a few seconds being used in

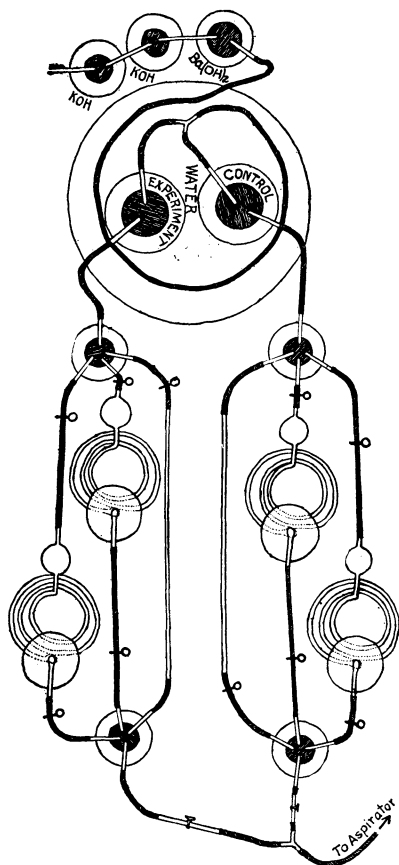


FIG. 1.

switching the currents of air. From the absorption tubes the air passes to another switch board bottle containing some H_2SO_4 . A tube directly from one switch board bottle to the other makes it possible to keep air passing through the cultures without using the absorption tubes; but this was found inadvisable and was largely given up, because when the absorption tube is returned to use, the height of liquid in it slackens the flow of air, and the irregularity so introduced is not immediately overcome by lengthening the siphon by an equal height of liquid. The glass stop cocks to which the air passes next regulate its rate of movement. These cocks must be selected by experience, and once they are working well must not be touched. The H_2SO_4 in the bottles below them prevents their obstruction by the precipitation of water.

As aspirators I have used barrels, and latterly a metal cask holding 200 liters, the vessel always having a lateral bung so that it might lie on its side. With this aspirator it was possible to run air at the speed I chose, usually about three liters per hour, through both cultures for over thirty hours. It was always filled (the filling requiring but few minutes) the last thing at night, and the effect of the interruption was lost in the long

interval before the next change of solutions. The volume of water in these aspirators was so great that the surface sank very slowly; and with a siphon over two meters long the loss of the effective pull was so slow that it could have had but little influence on the result if neglected; but even this I tried roughly to correct by lengthening the siphon as the day passed.

The siphon was a glass tube with the largest bore, almost 3^{mm}, with which the stream would be certain to carry down any bubble forming in it. The siphon could carry water from the aspirator so rapidly that the limited rate at which air enters the latter has no great influence on the pressure in it, and almost the whole pull of the siphon is exerted against each of the glass stop cocks. Any attempt to regulate the flow in the usual way, by restricting the siphon, reduces the suction in the aspirator; then the more rapid movement of air through one culture, the slower it is through the other. I have found it utterly impossible to balance the resistance in the two parts of the experiment so perfectly that they would keep the balance through the whole time of an experiment, except in the way described, by having the same high and steady suction act constantly on both.

The baryta water was put into the absorption tubes with the apparatus at the left of *fig. 2*. From the large bottle of stock solution it was allowed to run into the tube *A* until the fine tube in the top of this just broke the meniscus. The escape was then connected by a rubber joint with the small end of the absorption tube, and the liquid allowed to run out until the tube inserted in the lower end of *A* broke the meniscus; this measured out 100^{cc} without its coming in contact with any CO₂ except in the air in the absorption tube. Of course the stale baryta water standing in the escape of *A* was washed out just before the tube was filled. No baryta water was allowed to stand in the small bulb of the Winckler tubes.

After each interval of experiment the solution used was poured from the absorption tube into a bottle holding a little over 100^{cc}, without exposure to air except that in the bottle; and the bottle was immediately placed in the position of *B* in *fig. 2*, or else stoppered. If respiration had been active enough

to form a visible precipitate, this was allowed to settle, and the solution then siphoned into *C*. As one-third of the baryta water used was ordinarily more than was needed for titration, I was

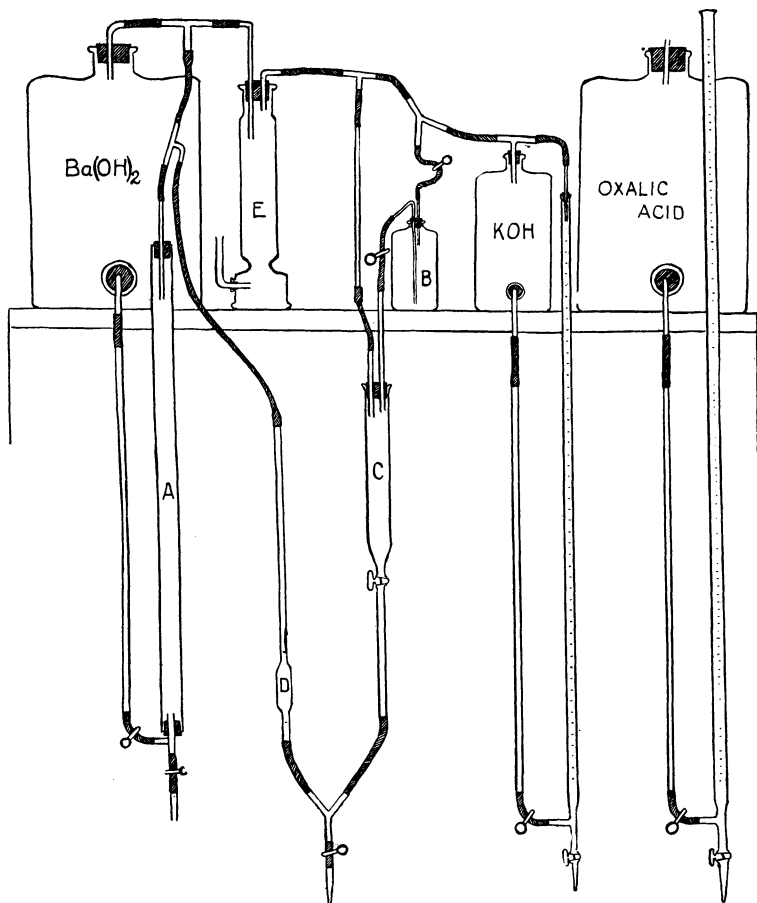


FIG. 2.

able to use it freely in washing out the apparatus before beginning to measure it. For the actual measurement I used the "burette" *D*, which I blew from heavy tubing with a bore of less than 3^{mm}; from the scratch above the bulb to that below is exactly 10^{cc}. The titrating was done in 100^{cc} bottles, with perforated corks. Two drops of neutral alcoholic phenolphthalein

were put in the bottle, which was then corked; 10^{cc} of the solution to be tested were run from *D*, whose outlet was stuck through the cork; then the outlet of the burette containing oxalic acid was stuck through the cork and enough run in to just neutralize. The acid was run in cautiously enough so that the color disappeared with the final addition of a single drop; this made it unnecessary to titrate back, though a burette of KOH of the proper strength was always ready for use if needed. As the diagram (*fig. 2*) shows, every vessel of any kind containing an alkaline solution can communicate with the air only through the tower *E*, containing pumice soaked in KOH.

For the idea of this method of titrating I am indebted to Symons and Stephens.^{4†} When, as in this work, extreme accuracy is necessary, titrating in a flask closed with the thumb, as is directed in manuals of quantitative analysis, yields serious irregularities; and when the liquid to be tested is measured by a pipette into which it is sucked, the results are not even tolerably uniform. Using the common method I often made five determinations of the titre to get a "reliable" average. The first two are likely to agree entirely, and almost never vary by more than one drop (0.05^{cc}); when they agree I call any more tests superfluous. This accuracy is the more notable because the acid used was $\frac{N}{100}$. The usual method of determining the CO₂, in milligrams, has no recommendation except custom; expressing it in volume of a normal solution is in much better harmony with modern chemical methods. My acid was practically 0.44, as strong as the most dilute one commonly used, of which 1^{cc} is the equivalent of 1^{mg} CO₂. It permits therefore more than twice the accuracy.

The limit of positive accuracy in volumetric analysis is one drop of the strongest solution in use. The directions extant for making up the baryta water provide for its being several times as strong as the acid it is titrated with. To get the benefit of the use of very dilute acid I have also used weak baryta water, not more than twice as strong, at the most, as the $\frac{N}{100}$ acid,

^{4†}SYMONS, W. H., and STEPHENS, F. R., Carbon dioxide. Its volumetric determination. Journ. Chem. Soc. London 69: 869-881. 1896.

except in experiments in which I anticipated a tremendous evolution of CO_2 . Such dilute baryta water still removes all CO_2 from the air.

As subjects of experiments in this work I have used Cardamine, Callitriche, Ceratophyllum, Potamogeton, Elodea, tadpoles, and fish (an immature cyprinoid, not determined). Elodea and tadpoles were the subjects of extended series of experiments, the other plants and the fish being used but a few times, to be sure of the general validity of the results.

In three changes of residence since doing the work with tadpoles I have misplaced the detailed results of the individual experiments on them. The solutions used on them were all dilute enough not to be fatal during the time of experiment, and their results agree in all essentials with those yielded by similar work on Elodea. Single experiments with the chlorids of gold and platinum on tadpoles failed to show any influence on the evolution of CO_2 , probably because they were used in too great dilution. With CoCl_2 the evolution of CO_2 was accelerated. The other chemicals employed, Zn, Cu, Cd, and Hg, were used again on Elodea. Because of this, and of the question I will present shortly as to the significance of any of the results with strong metallic poisons, I do not think it worth while to hunt up and publish the detailed results on tadpoles. Nor will I consume space with any considerable part of the detailed results on other subjects. In the work with animal subjects, solutions much too weak to kill them stimulated them to a visibly increased muscular activity. This was most pronounced, with relation to the toxicity of the several metals, with Cd, a little less by Cu, and least of all the substances used by Pt, in which the tadpoles were very sluggish, in spite of the acidity of the " PtCl_4 " employed. It seemed to me, from a comparison of the results obtained at the same time on tadpoles and on Callitriche, that the excited muscular activity of the former did not produce a relatively greater evolution of CO_2 ; from which I concluded that the protoplasm of both was stimulated equally, the plant's response being, for obvious reasons, less conspicuous.

A study of the acceleration of respiration would be blind

reading, without accompanying information on the toxicity of the chemicals used. By the fragmentary condition of our knowledge on this subject, I have been obliged to determine for myself the toxicity of these substances for the different organisms, and have compiled tables on the tadpole, Callitriche, and Elodea, of which the two former may be omitted, with the corresponding statistics on respiration. These experiments on Elodea were made with young growing tips, 2-4^{cm} long. The condition of the plant, dead or alive, was determined by trying to plasmolyze the youngest grown leaves in KNO_3 . Relieved of details as to concentrations which do not seriously injure or are stronger than enough to kill, the results follow, the figures being the number of liters of solution in which one gram-molecule is imagined distributed.

$\text{Pb}(\text{NO}_3)_2$	Alive but hardly sound after 24 hours in $\frac{\text{M}}{10}$. Appears normal in $\frac{\text{M}}{25}$.
KCN	Dead in 30 hours in $\frac{\text{M}}{100}$, but alive in $\frac{\text{M}}{200}$.
KOH	Dead in 2 hours in $\frac{\text{M}}{100}$: in one test dead, in another almost so after 24 hours in $\frac{\text{M}}{200}$.
NiCl_2	Dead in 24 hours in $\frac{\text{M}}{100}$: somewhat unsound in $\frac{\text{M}}{200}$.
ZnSO_4	Dead in three hours in $\frac{\text{M}}{100}$: alive but unsound after 26 hours in $\frac{\text{M}}{200}$: sound indefinitely in $\frac{\text{M}}{500}$, below 20° C.
HCl	Dead in 15 hours in $\frac{\text{M}}{500}$: unsound after 24 hours in $\frac{\text{M}}{1000}$.
CoCl_2	Practically dead after 24 hours in $\frac{\text{M}}{10,000}$.
$\text{Cd}(\text{NO}_3)_2$	Almost dead after 24 hours in $\frac{\text{M}}{20,000}$: sound in $\frac{\text{M}}{40,000}$.
CuSO_4	Dead in 25 hours in $\frac{\text{M}}{20,000}$: alive in $\frac{\text{M}}{40,000}$.
HgCl_2	Sound after 5 hours in $\frac{\text{M}}{40,000}$, but dead in 30 hours in $\frac{\text{M}}{80,000}$.
AgNO_3	Dead in 24 hours in $\frac{\text{M}}{160,000}$: unsound in $\frac{\text{M}}{320,000}$.

These determinations were made at a temperature of about 20° C.

K is a shade less poisonous than Pb; Na, a little less still, compared as nitrates. With the tadpoles, Pb and Cd were more

toxic than with Elodea, but Co is less so. PtCl_4 was not more toxic than could be ascribed to its acidity; gold chlorid was about equally so; and uranyl (nitrate) was about as toxic as Co. KCN was not tested on tadpoles, but would certainly not have been found harmless as with Elodea. My KCN was strictly *p. c.*, and was about 0.7 as alkaline as equivalent KOH. Their toxicity toward Elodea is about proportional to their alkalinity.

The modern work on the toxicity of inorganic salts to higher plants is not as conclusive and satisfactory in its botanical as in its chemical aspects. At least in large part it has been done without reference to the temperature. But I have found, as was *a priori* reasonable, that at 28°C. , and still more at 37.5°C. (at which the temperature alone is fatal in time), Elodea succumbs much more readily than below 20°C. If an arbitrary time limit, say 24 hours, is placed on the experiment, it is evident that the higher the temperature the more toxic the substances tested will appear. Beside this, I have some evidence, though not enough to be conclusive, that at higher temperatures the adaptation to the poison does not increase in rapidity in proportion to the injurious action; when this is true, the plant may become adapted and thrive in a solution at a lower temperature, which at a higher would kill it.

The range of variation in apparently reliable statements as to the toxicity of the same substances with different organisms is nothing short of astounding. Kahlenberg and True place Cu, Fe, Ni, and Co on a par, as to their action on *Lupinus albus*. Heald⁴² finds Ni and Hg equally toxic to *Zea*. Coupin⁴³ says CuSO_4 diluted to 1 (mol.?) in 700,000,000 checks the growth of wheat, while AgNO_3 acts likewise when 1 in 1,000,000, and

⁴²HEALD, F. D., On the toxic effect of dilute solutions of acids and salts upon plants. BOT. GAZ. 22: 125-153. 1896.

⁴³COUPIN, H., Sur la sensibilité des végétaux supérieurs à des doses très faibles des substances toxiques. Comptes Rendus, Paris 132: 645-647. 1901. Coupin claims priority for the discovery that distilled water is not in itself a poison, having published it in 1900 (Cf. de Clèves, L'eau distillée, est-elle pure? in La Nature 28 1: 196. 1900), and DEHÉRAIN and DEMOUSSY (Compt. Rend. 132: 523-527. 1901), having repeated it. The fact was not new by some years when Kahlenberg and I did our work in 1898 (published in 1899).

$\text{Pb}(\text{NO}_3)_2$ when 1 in 100,000. From the Pb and Ni results Elodea (and Callitriche) would seem very resistant as compared with other flowering plants; and in fact the tabulated results as a whole justify that view. And yet it ranks with the most sensitive when treated with Hg or Ag.

[*To be concluded.*]